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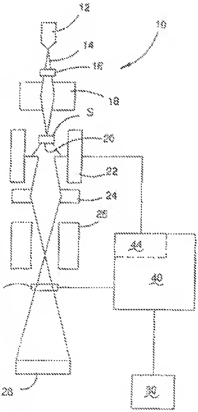
(54) Title: PHASE RETRIEVAL FROM FOCUSED AND DEFOCUSED ELECTRON BEAM IMAGES



(57) Abstract: Apparatus and method for producing a phase image by irradiating an object with electrons is disclosed. The method and apparatus include producing in focus and defocused images of the object by focusing an electron beam with a magnetic lens and controlling the magnetic lens so as to adjust the position of the focus so as to produce the in focus and defocused images. The defocused images are processed to remove distortions introduced when the defocused images are produced by transforming the defocused images in accordance with a transformation to compensate for those distortions. The transformation may be in accordance with a transformation determined by imaging a known reference grid and determining a transformation to return that grid to its known position, or by comparing the defocused images with the in focus image, and using the in focus image as the reference for determining a transformation to transform the defocused images.

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PHASE RETRIEVAL FROM FOCUSED AND DEFOCUSED ELECTRON BEAM IMAGES

Background of the Invention

This invention relates to an apparatus and method for imaging an object and to a transmission electron microscope which includes the apparatus and which can perform the method. The invention has particular application to the production of phase images of an object and which are produced by a transmission electron microscope.

Background of the Invention

It is known that a phase image of an object can be generated by quantitative determination of the phase of the radiation wavefield emanating from the object. In International Patent Application No. PCT/AU99/00949 (Publication No. WO 00/26622) owned by The University of Melbourne, a method and apparatus for producing phase images is disclosed which involves solving the transport of intensity equation to enable both phase and intensity data relating to the object to be determined independently. This enables a phase image of an object to be produced which can provide detail, particularly in biological samples, which is not apparent when a conventional intensity or absorption image of the object is viewed. The contents of the above-mentioned International specification are incorporated into this specification by this reference.

The above International application is primarily concerned with generating images in the visible part of the spectrum, although the algorithm disclosed in the application is also useful for producing images from other parts of the electro-magnetic spectrum including X-rays and also from particles exhibiting wavelike phenomena including electrons, neutrons and atoms.

Summary of the Invention

The present invention is particularly concerned with the generation of phase images by irradiating an object with electrons and to such phase images produced by a transmission electron microscope.

The invention, in a first aspect, may be said to reside in apparatus for producing a phase image of an object, including:

means for producing a beam of electrons;

support means for supporting the object;

at least one magnetic lens for receiving an electric signal and producing a magnetic field for focusing the electron beam emanating from the object;

control means for controlling the electric signal applied to the magnetic lens so as to adjust the position of focus of the beam relative to a prescribed plane so that an in focus image and/or defocused images of the object are produced at the plane; and

detecting means for detecting the images to enable a phase image of the object to be generated.

According to this aspect of the invention the images required in order to generate the phase image are produced by controlling the magnetic lens so that the magnetic lens is adjusted in magnetic strength to thereby produce in focus and/or defocused images at the plane, which provide the data from which the phase image can be generated.

Preferably the at least one lens is an objective lens.

Preferably the magnetic lenses or magnetic lens are formed from metal grids or plates to which a voltage is applied to generate a magnetic field which focuses the electron beam.

Preferably the detector comprises a charge coupled device

at the plane for detecting the electron beam and providing a data output indicative of the images detected by the charge coupled device.

Preferably the charge coupled device is connected to processing means for receiving the data and for processing the data to form a phase image.

Preferably the processing means processes data in accordance with the algorithm disclosed in the above-identified International patent application to produce the phase image.

The control means may comprise a manual control for adjusting the voltage applied to the magnetic lens.

However, in the preferred embodiment of the invention the control means forms part of the processing means and the processing means, upon user input, causes the voltage supplied to the lens to be adjusted so as to produce in focus and/or defocused images of the object to provide the data from which the phase image can be generated.

Preferably the processor also compensates for aberrations introduced by the magnetic lens by performing a transformation on the data provided by the detector relating to each of the images.

In one embodiment the transformation is obtained by obtaining data relating to a known reference sample, producing a transformation required to convert the detected data relating to the known reference sample to conform with the known reference sample, applying that transformation to the data produced by the detector so as to convert the data to thereby compensate for aberrations introduced by the magnetic lens.

In another embodiment of the invention the transformation is obtained by comparing the defocused image with the in focus image, and the defocused image is transformed so as to align data in the defocused image with corresponding data in the in focus image.

Preferably the transformation comprises a coarse transformation in which the defocused image and the in focus image are overlapped and one of the images is moved relative to the other image to align data in the images, and then a fine alignment step in which the defocused image is divided into cells and each cell is compared with a corresponding cell of the in focus image, so as to move data in each cell to more finely align that data with the data in corresponding cells of the in focus image.

The coarse alignment step may further include cropping the images so as to provide an image consisting of the overlapping regions of the defocused and in focus images, and wherein the fine alignment step includes dividing the cropped defocused images into the plurality of cells.

Preferably the plurality of cells of the defocused image are moved relative to the in focus cells in a raster pattern, and correlation values for each movement in the pattern are obtained to enable the best correlation, and therefore the location of the in focus cell relative to the defocused cell which provides the best correlation.

Preferably the correlation value for each cell is averaged with neighbouring cells so as to produce an average correlation factor.

Preferably the cells are warped in accordance with the correlation factors in order to move information within the cells so as to provide fine correlation of that information with the corresponding information in the in

focus image.

Preferably the apparatus is included in a transmission electron microscope and the transmission electron microscope further includes:

an anode for attracting electrons from the electron source;

at least one condenser lens for receiving an electron beam from the anode and focusing the electron beam into a relatively thin beam for application to the object;

the objective lens for receiving electrons transmitted through the object and for focusing the electron beam into an image; and

an intermediate lens and a projector lens for imaging the electron beam onto the detector.

The invention may also be said to reside in a method of producing a phase image of an object from an electron beam, including:

causing an electron beam to impinge on an object and detecting the electron beam emanating from the object;

focusing the electron beam emanating from the object with a magnetic focus lens by adjusting an electric signal supplied to the lens so as to produce an in focus and/or defocused images of the object;

processing data relating to the in focus and/or defocused images of the object so as to generate a phase image of the object.

Preferably the at least one magnetic lens is an objective lens.

Preferably the magnetic lens is formed from metal coils, grids or plates to which a voltage is applied to generate a magnetic field which focuses the electron beam.

Preferably the detector comprises a charge coupled device for detecting the electron beam and providing a data output indicative of the images detected by the charge coupled device.

Preferably the detector is connected to processing means for receiving the data and for processing the data to form a phase image.

Preferably the processing step processes data in accordance with the algorithm disclosed in the above-identified International patent application to produce the phase image.

The control of the magnetic lens may comprise a manual control for adjusting the voltage applied to the magnetic lens.

However, in the preferred embodiment of the invention the control is performed under the influence of a processing means and the processing means, upon user input, causes the voltage supplied to the lens to be adjusted so as to produce in focus and/or defocused images of the object to provide the data from which the phase image can be generated.

Preferably the processor also compensates for aberrations introduced by the magnetic lens by performing a transformation on the data provided by the detector relating to each of the images.

Preferably the transformation is obtained by obtaining data relating to a known reference sample, producing a transformation required to convert the detected data relating to the known reference sample to conform with the known reference sample, applying that transformation to the data produced by the detector so as to convert the

data to thereby compensate for aberrations introduced by the magnetic lens.

In another embodiment of the invention the transformation is obtained by comparing the defocused image with the in focus image, and the defocused image is transformed so as to align data in the defocused image with corresponding data in the in focus image.

Preferably the transformation comprises a coarse transformation in which the defocused image and the in focus image are overlapped and one of the images is moved relative to the other image to align data in the images, and then a fine alignment step in which the defocused image is divided into cells and each cell is compared with a corresponding cell of the in focus image, so as to move data in each cell to more finely align that data with the data in corresponding cells of the in focus image.

The coarse alignment step may further include cropping the images so as to provide an image consisting of the overlapping regions of the defocused and in focus images, and wherein the fine alignment step includes dividing the cropped defocused images into the plurality of cells.

Preferably the plurality of cells of the defocused image are moved relative to the in focus cells in a raster pattern, and correlation values for each movement in the pattern are obtained to enable the best correlation, and therefore the location of the in focus cell relative to the defocused cell which provides the best correlation.

Preferably the correlation value for each cell is averaged with neighbouring cells so as to produce an average correlation factor.

Preferably the cells are warped in accordance with the

correlation factors in order to move information within the cells so as to provide fine correlation of that information with the corresponding information in the in focus image.

The invention also provides an apparatus for producing a phase image of an object using in focus and defocused images produced by a beam of electrons which transmit through or are reflected from an object, and in which the beam of electrons is controlled to produce in focus and defocused images of the object, said apparatus including:

processing means for;

(a) removing distortions from the defocused images introduced into the defocused images when the defocused images are obtained by the control of the magnetic lens; and

(b) processing the in focus and defocused images to produce a phase image of the object.

Preferably the phase image is produced from the in focus image and two defocused images of the object.

In another embodiment of the invention the transformation is obtained by comparing the defocused image with the in focus image, and the defocused image is transformed so as to align data in the defocused image with corresponding data in the in focus image.

Preferably the transformation comprises a coarse transformation in which the defocused image and the in focus image are overlapped and one of the images is moved relative to the other image to align data in the images, and then a fine alignment step in which the defocused image is divided into cells and each cell is compared with a corresponding cell of the in focus image, so as to move data in each cell to more finely align that data with the

data in corresponding cells of the in focus image.

The coarse alignment step may further include cropping the images so as to provide an image consisting of the overlapping regions of the defocused and in focus images, and wherein the fine alignment step includes dividing the cropped defocused images into the plurality of cells.

Preferably the plurality of cells of the defocused image are moved relative to the in focus cells in a raster pattern, and correlation values for each movement in the pattern are obtained to enable the best correlation, and therefore the location of the in focus cell relative to the defocused cell which provides the best correlation to be obtained.

Preferably the correlation value for each cell is averaged with neighbouring cells so as to produce an average correlation factor.

Preferably the cells are warped in accordance with the correlation factors in order to move information within the cells so as to provide fine correlation of that information with the corresponding information in the in focus image.

In one embodiment the transformation is obtained by obtaining data relating to a known reference sample, producing a transformation required to convert the detected data relating to the known reference sample to conform with the known reference sample, applying that transformation to the data produced by the detector so as to convert the data to thereby compensate for aberrations introduced by the magnetic lens.

The invention also provides an apparatus for producing a phase image of an object using in focus and defocused

images produced by a beam of electrons which transmit through or are reflected from an object, and in which the beam of electron is controlled to produce in focus and defocused images of the object, said apparatus including:

processing means for;

(a) removing distortions from the defocused images introduced into the defocused images when the defocused images are obtained by the control of the magnetic lens; and

(b) processing the in focus and defocused images to produce a phase image of the object.

Preferably the phase image is produced from the in focus image and two defocused images of the object.

Brief Description of the Drawings

A preferred embodiment of the invention will be described, by way of example, with reference to the accompanying drawings in which:

Figure 1 is a view of an apparatus embodying the invention;

Figure 2 is a view of a reference grid used in the preferred embodiment of the invention;

Figure 3 is a view of a reference grid as detected by the apparatus of Figure 1;

Figure 4 is a view of an actual sample as detected by the apparatus;

Figure 5 is a view of an actual sample with aberrations removed according to the preferred embodiment of the invention;

Figures 6, 7 and 8 are flow charts illustrating a further embodiment; and

Figures 9, 10, 11, 12, 13 and 14 are diagrams to assist understanding of the flow charts of Figures 6 to 8.

Detailed Description of the Preferred Embodiments

An apparatus 10 according to the preferred embodiment of the invention is shown which is basically in the form of a transmission electron microscope. The apparatus 10 includes an electron gun 12 for producing a beam of electrons 14. The beam of electrons 14 is attracted towards an anode 16 by a positive voltage applied to the anode 16. The beam 14 is focused into a thin coherent beam by a condenser lens 18. The condenser lens may include a number of separate lenses, but only one lens is shown simply for convenience.

A sample *S* is located on a support 20 in the beam 14 which emanates from the condenser lens 18 so that the beam of electrons is able to penetrate the sample *S*. The beam emanating from the sample *S* is focused by an objective lens 22 which may include a physical aperture to restrict the electron beam passing through the objective lens 22.

An intermediate lens 24 and a projector 26 are provided for further conditioning of the electron beam and for imaging the electron beam on a fluorescent screen 28.

The fluorescent screen 28 enables an image of the sample S to be observed.

The apparatus 10 includes a processing section 40 which is connected to the charge coupled device 27 for receiving data relating to the image and for displaying the image on the monitor 30 if required. The charge coupled device 27 can be moved out of the field of view of the fluorescent screen 28 to enable the screen 28 to display an image.

The processing section 40 also includes a control section 44 which is connected to the objective lens 22 for supplying voltages to the objective lens so as to control the objective lens 22. The control section 44 may also be coupled to the other magnetic lenses 18, 24 and 26 and

also the anode 16 for supply of appropriate control voltages so that the beam 14 is focused and conditioned for imaging onto the device 28.

In general, a user is able to control the condenser lens 18 by adjusting a control knob manually and the adjustment of the condenser lens 18 determines the "spot size" of the electron beam impinging on the sample S. If the condenser lens includes a second lens, the second lens can also be controlled by a user actuated knob which controls the intensity or brightness by changing the size of the spot on the sample from a wide dispersed spot to a pinpoint beam.

In order to produce a phase image of the object as distinct from a conventional "electron intensity or absorption image" which would normally be what is observed on the device 27, at least two images (preferably three images) of the sample S are required. The images comprise either an in focus image and one defocused image of the sample or two defocused images of the sample. The in focus and defocused images are obtained by adjusting the control voltage supplied by the control unit 44 to the objective lens 22 so as to change the magnetic field generated by the lens 22 to, in turn, change the focus produced by that lens so that the image is focused on the screen 28 to produce an in focus image or, alternatively, images are focused on either side of the plane of the device 27 to produce defocused images of the sample S.

In order to produce the in focus and/or defocused images the processing section 40 is controlled by a user input so as to produce the images required to generate a phase image of the sample. This can be done simply by appropriate input into the processing section 40 and the processing section 40 under the influence of appropriate software loaded into the processing section 40 can then

cause the control section 44 to output appropriate voltages to the objective lens 22. Changing the voltages will first cause the objective lens 22 to produce an in focus image of the sample and then two defocused images of the sample, one on each side of the focus plane at which the device 27 is located. As made clear above, rather than produce one in focus and two defocused images, two defocused images could be produced which will provide sufficient information to enable the phase image to be generated by the processing section 40.

The data collected by the device 27 is then supplied to the processing section 40 so that the data can be processed in accordance with the algorithm described in the above-mentioned International application and the phase image generated and displayed on the monitor 30.

The manipulation of the magnetic lenses will introduce significant aberrations into the images which are imaged onto the screen 28. In particular, the adjustment of the objective lens 22 to form defocused images will cause aberrations to be generated in those images which must be taken account of in order to produce a true phase image of the sample.

In order to compensate for aberrations introduced by a change in the magnetic focusing power of the lens 22, a transformation is generated which is applied to the data received by the device 27 to transform that data so as to remove the aberrations introduced by the magnetic lens 22.

In order to generate the transformation a known reference grid such as that shown in Figure 2 is located on the support 20 in place of the sample S. An image of the reference grid 2 is then detected by the fluorescent screen 28 with a particular voltage applied to the lens 22 to generate, for example, a defocused image. The image

detected by the screen 28 is that shown in Figure 3 and it can be seen that the image has been distorted somewhat by the aberrations introduced by the change in the magnetic lens 22.

Since the processor 40 can be provided with data relating to the true configuration and shape of the reference grid shown in Figure 2, a comparison between the detected reference grid as shown in Figure 3 and the true grid as shown in Figure 2 can be made and a transformation generated which will transfer the data of Figure 3 into the position shown in Figure 2 to remove the aberration. The transformation which is required to transform the image of Figure 3 back to the true reference image of Figure 2 is therefore stored for use in relation to a sample. Another transformation is generated for a different voltage applied to the lens 22 to produce either a different defocused image or, for that matter, an in focus image of the reference sample.

When an actual sample is detected by the device 27, as for example shown in Figure 4, the sample will have aberrations introduced into it by the change in magnetic power of the lens 22 in order to produce the defocused images or, for that matter, the in focus image as described above. The transformation which has previously been generated is therefore applied to the data relating to the image of Figure 4 so as to transform that image in accordance with the transformation to thereby produce a true image without the aberrations.

Such an image is shown in Figure 5 which has the aberrations removed to thereby provide a true image of the sample.

Similarly, the other images which are created are also transformed in accordance with the particular

transformation relating to that image (ie. which relates to the voltage applied to the lens 22 to produce the image) so that the aberrations in that image are removed.

The data relating to the image is therefore free of aberrations and can be processed to provide a true phase image of the actual sample free of aberrations created by the change in magnetic power of the objective lens 22.

Figures 6 to 8 show a further embodiment of the manner in which the system compensates for distortions introduced when the defocused images are obtained.

Figure 6 is an overview of the system according to this embodiment. Defocused images 101 and 102 and in focus image 103 are obtained in the manner described above. The system may include an automated software routine shown at step 105 which performs an automatic coarse alignment of the three images with a view to placing the information in the images in the same position or, in other words, overlapping the information in the images. However, in the preferred form of this aspect of the invention, this step is performed as a manual alignment step as shown by step 107. After the coarse alignment has been performed in accordance with steps 105 or 107 (or both of those steps in combination), a fine alignment step 106 is performed to provide fine alignment of the images so that the images can then be used to form the phase image of the sample in the manner previously described.

Figure 7 is a flow chart which shows the detail of the manual alignment step 107 of Figure 6.

As previously described, in the course of making the defocused images, the change in the potentials applied to the lens 22 will distort the defocused images, thereby resulting in the information in the images not being the

in same position as the in focus image, which makes it impossible or greatly reduces the quality of the phase image which will be obtained from those three images. The slight distortion of the three images is represented in Figure 9. The images 101 and 102 are slightly twisted (in opposite directions relative to the image 103).

At step 110 the images 101, 103 and 102 are laid one above the other to form an image stack. Thus, this places the images in overlapping configuration one above the other, as shown in Figure 10. As is shown in Figure 10, the information contained in the images does not completely align because of the distortions referred to above.

At step 111 the images are rescaled if necessary simply to make the images more easy to handle. This is obviously desirable if the original images 101, 102 and 103 are relatively large.

At step 112 the in focus image is selected and one of the defocused images is selected. These images are in the overlapped configuration previously described.

At step 113 the user manually manoeuvres the in focus image so that the information in the two images is placed into alignment. This is done by click and drag procedures on the user's PC, which translate, rotate and stretch one of the images to place the information in the images into alignment. In general, this will result in slight rotation, translation or scaling of one of the images relative to the other.

As shown in Figure 11, the three images 101, 102 and 103 are shown in stacked or overlapped configuration, wherein the images 102 and 103 have been rotated or moved so that the information in each of the images is in alignment, as is shown in Figure 11.

At step 114 the user makes a decision that the alignment of the two images is correct so that the information in the images coincides. The program then returns to step 112 and the other defocused image is aligned in the same manner with the in focus image.

At step 115 the original defocused images 101 and 102 are transformed using the same alignment parameters which were obtained at step 113. Thus, the defocused images are transformed relative to the in focus image to place the information in each of the three images into as exact as possible alignment the operator can obtain by viewing the images in overlapped configuration.

When the images are aligned, the images are cropped at step 116. This is done by identifying the largest possible rectangle 200 (see Figure 12) within the complete overlap of the images obtained at step 115 so as to exclude parts of each of the images which fall outside the common rectangle.

This then completes the coarse alignment of the three images.

Figure 8 shows the fine alignment which is used to align more finely the information within the three images obtained after the coarse alignment has been performed in accordance with Figure 8. This fine alignment step is performed automatically by the processor 40 in accordance with the algorithm described with reference to Figure 8.

With reference to Figure 8, the in focus image 103 and one of the defocused images (such as the image 101) are selected so as to perform the fine alignment on the defocused image 101 relative to the in focus image 103.

At step 117 the cropped image 101 from step 116 is divided into a plurality of cells 202 (see Figure 13). That is, a plurality of separate regions, each of which will be processed individually in order to perform alignment of the information in the cells with the corresponding cells in the in focus image 103.

At step 118 the information in the cell 202 of the defocused image is correlated with the information in the cell 203 of the in focus image at each point of a 20x20 pixel scan area. In other words, the defocused cell is placed over the in focus cell and the defocused cell moved relative to the in focus cell in a 20x20 pixel raster pattern (see Figure 14).

At step 119 a relative score for the degree of correlation at each position in the 20x20 pixel scan is obtained. The purpose of this step is to identify the position of the defocused cell relative to the in focus cell which yields the best correlation of the information in that cell relative to the information in the in focus cell, and therefore the best alignment. For example, for each of the locations of the defocused cell in the 20x20 pixel raster pattern scan, a relative score of the correlation between the information in the cell at that position relative to the information in the in focus image is obtained. For example, the score can be a value between 0 and 1. The correlations are stored in memory within the processor 40. This procedure continues for each of the cells of the defocused image until all of the cells have been correlated. This part of the program then ceases at step 120.

The other defocused image 102 is then selected and the same procedure occurs, as described with reference to steps 117 to 119, so that stored correlation values are obtained for each of the cells of the second defocused

image. The best correlation for each cell position is therefore provided by the highest stored correlation value for each of the cells in the stored values. At step 121, each correlation for each cell is multiplied by the correlation value for its neighbouring cells to perform an averaging process. The purpose of the averaging process is to remove possible irregularities where very little information is contained in a particular cell and spurious information points in that cell may result in a correlation value which is contrary to the correlation value obtained in neighbouring cells. For example, if there is a large amount of information in a particular cell, and a very high degree of correlation between that information in the in focus cell, then the averaging process will have very little impact on the correlation value obtained for that cell. However, if there is very little information in a particular cell and a correlation value obtains a result which would tend to want to shift that particular cell in a direction or manner contrary to the manner in which surrounding cells are to be shifted, the averaging process will tend to smooth that shift and bring it more into alignment with the general trend of movement required in neighbouring cells in order to align the information in those cells with the information in the in focus image.

At step 122 the peak value in each correlation map is obtained and the offset of that peak correlation from the centre of the cell is stored in a correlation table. This provides a general indication of the amount the defocused cell should be moved relative to the in focus cell in order to provide correlation between the information in each cell.

At step 123, the defocused image is warped in accordance with the information in the correlation table obtained at step 122 to effectively move the defocused cells by a

certain amount in order to provide correlation between the information in those cells with the corresponding information in the in focus cells of the in focus image 103. For each pixel in the final destination image a contribution from the appropriate pixels in the source image is computed based upon the values for the warping matrix. Therefore there are no 'cell boundaries' per se, the warp is really a continuously variable stretch operation. This therefore performs a fine alignment of the information in each image after the coarse alignment, so as to provide much better correlation of the information in the defocused images and the in focus image which can then be used to provide the data for producing the phase image in accordance with the algorithm previously described in the aforementioned co-pending International application.

Thus, this embodiment of the invention, rather than using a separate grid or reference value in order to obtain a measure of the amount of distortion introduced by the magnetic lens 22 when the in focus and defocused images are obtained, uses the in focus image as the perfect image, and therefore uses that image as the reference to obtain an effective transformation which will transform the defocused image to compensate for the distortions and aberrations introduced by the lens 22 when the defocused images are obtained.

Since modifications within the spirit and scope of the invention may readily be effected by persons skilled within the art, it is to be understood that this invention is not limited to the particular embodiment described by way of example hereinabove.

Claims

1. Apparatus for producing a phase image of an object, including:

means for producing a beam of electrons;

support means for supporting the object;

at least one magnetic lens for receiving an electric signal and producing a magnetic field for focusing the electron beam emanating from the object;

control means for controlling the electric signal applied to the magnetic lens so as to adjust the position of focus of the beam relative to a prescribed plane so that an in focus image and/or defocused images of the object are produced at the plane; and

detecting means for detecting the images to enable a phase image of the object to be generated.

2. The apparatus of claim 1 wherein the at least one lens is an objective lens.

3. The apparatus of claim 1 wherein the magnetic lenses or magnetic lens are formed from metal grids or plates to which a voltage is applied to generate a magnetic field which focuses the electron beam.

4. The apparatus of claim 1 wherein the detector comprises a charge coupled device at the plane for detecting the electron beam and providing a data output indicative of the images detected by the charge coupled device.

5. The apparatus of claim 4 wherein the charge coupled device is connected to processing means for receiving the data and for processing the data to form a phase image.

6. The apparatus of claim 5 wherein the processing

means processes data in accordance with an algorithm which solves the transport of intensity equation to produce the phase image.

7. The apparatus of claim 5 wherein the control means forms part of the processing means and the processing means, upon user input, causes the voltage supplied to the lens to be adjusted so as to produce in focus and/or defocused images of the object to provide the data from which the phase image can be generated.

8. The apparatus of claim 5 wherein the processor also compensates for aberrations introduced by the magnetic lens by performing a transformation on the data provided by the detector relating to each of the images.

9. The apparatus of claim 8 wherein the transformation is obtained by obtaining data relating to a known reference sample, producing a transformation required to convert the detected data relating to the known reference sample to conform with the known reference sample, applying that transformation to the data produced by the detector so as to convert the data to thereby compensate for aberrations introduced by the magnetic lens.

10. The apparatus of claim 8 wherein the transformation is obtained by comparing the defocused image with the in focus image, and the defocused image is transformed so as to align data in the defocused image with corresponding data in the in focus image.

11. The apparatus of claim 10 wherein the transformation comprises a coarse transformation in which the defocused image and the in focus image are overlapped and one of the images is moved relative to the other image to align data in the images, and then a fine alignment

step in which the defocused image is divided into cells and each cell is compared with a corresponding cell of the in focus image, so as to move data in each cell to more finely align that data with the data in corresponding cells of the in focus image.

12. The apparatus of claim 11 wherein the coarse alignment step further includes cropping the images so as to provide an image consisting of the overlapping regions of the defocused and in focus images, and wherein the fine alignment step includes dividing the cropped defocused images into the plurality of cells.

13. The apparatus of claim 12 wherein the plurality of cells of the defocused image are moved relative to the in focus cells in a raster pattern, and correlation values for each movement in the pattern are obtained to enable the best correlation, and therefore the location of the in focus cell relative to the defocused cell which provides the best correlation to be obtained.

14. The apparatus of claim 13 wherein the correlation value for each cell is averaged with neighbouring cells so as to produce an average correlation factor.

15. The apparatus of claim 14 wherein the cells are warped in accordance with the correlation factors in order to move information within the cells so as to provide fine correlation of that information with the corresponding information in the in focus image.

16. A transmission electron microscope including the apparatus of claim 2 wherein the transmission electron microscope further includes:

an anode for attracting electrons from the electron source;

at least one condenser lens for receiving an

electron beam from the anode and focusing the electron beam into a relatively thin beam for application to the object;

the objective lens for receiving electrons transmitted through the object and for focusing the electron beam into an image; and

an intermediate lens and a projector lens for imaging the electron beam onto the detector.

17. A method of producing a phase image of an object from an electron beam, including:

causing an electron beam to impinge on an object and detecting the electron beam emanating from the object;

focusing the electron beam emanating from the object with a magnetic focus lens by adjusting an electric signal supplied to the lens so as to produce an in focus and/or defocused images of the object;

processing data relating to the in focus and/or defocused images of the object so as to generate a phase image of the object.

18. The method of claim 17 wherein the at least one magnetic lens is an objective lens.

19. The method of claim 18 wherein the magnetic lens is formed from metal coils, grids or plates to which a voltage is applied to generate a magnetic field which focuses the electron beam.

20. The method of claim 17 wherein the beam is imaged onto a detector which comprises a charge coupled device for detecting the electron beam and providing a data output indicative of the images detected by the charge coupled device.

21. The method of claim 17 wherein the processing step processes data in accordance with the algorithm

disclosed in the above-identified International patent application to produce the phase image.

22. The method of claim 17 wherein the focusing is performed under the influence of a processing means and the processing means, upon user input, causes the voltage supplied to the lens to be adjusted so as to produce in focus and/or defocused images of the object to provide the data from which the phase image can be generated.

23. The method of claim 17 wherein the processing step also compensates for aberrations introduced by the magnetic lens by performing a transformation on the data provided by the detector relating to each of the images.

24. The method of claim 23 wherein the transformation is obtained by obtaining data relating to a known reference sample, producing a transformation required to convert the detected data relating to the known reference sample to conform with the known reference sample, applying that transformation to the data produced by the detector so as to convert the data to thereby compensate for aberrations introduced by the magnetic lens.

25. The method of claim 23 wherein the transformation is obtained by comparing the defocused image with the in focus image, and the defocused image is transformed so as to align data in the defocused image with corresponding data in the in focus image.

26. The method of claim 25 wherein the transformation comprises a coarse transformation in which the defocused image and the in focus image are overlapped and one of the images is moved relative to the other image to align data in the images, and then a fine alignment step in which the defocused image is divided into cells and each cell is compared with a corresponding cell of the in focus image,

so as to move data in each cell to more finely align that data with the data in corresponding cells of the in focus image.

27. The method of claim 26 wherein the coarse alignment step further includes cropping the images so as to provide an image consisting of the overlapping regions of the defocused and in focus images, and wherein the fine alignment step includes dividing the cropped defocused images into the plurality of cells.

28. The method of claim 27 wherein the plurality of cells of the defocused image are moved relative to the in focus cells in a raster pattern, and correlation values for each movement in the pattern are obtained to enable the best correlation, and therefore the location of the in focus cell relative to the defocused cell which provides the best correlation to be obtained.

29. The method of claim 28 wherein the correlation value for each cell is averaged with neighbouring cells so as to produce an average correlation factor.

30. The method of claim 29 wherein the cells are warped in accordance with the correlation factors in order to move information within the cells so as to provide fine correlation of that information with the corresponding information in the in focus image.

31. An apparatus for producing a phase image of an object using in focus and defocused images produced by a beam of electrons which transmit through or are reflected from an object, and in which the beam of electron is controlled to produce in focus and defocused images of the object, said apparatus including:

processing means for;

(a) removing distortions from the defocused

images introduced into the defocused images when the defocused images are obtained by the control of the magnetic lens; and

(b) processing the in focus and defocused images to produce a phase image of the object.

32. The apparatus of claim 31 wherein the phase image is produced from the in focus image and two defocused images of the object.

33. The apparatus of claim 31 wherein the processing means is for removing distortions by a transformation obtained by comparing the defocused image with the in focus image, and the defocused image is transformed so as to align data in the defocused image with corresponding data in the in focus image.

34. The apparatus of claim 33 wherein the transformation comprises a coarse transformation in which the defocused image and the in focus image are overlapped and one of the images is moved relative to the other image to align data in the images, and then a fine alignment step in which the defocused image is divided into cells and each cell is compared with a corresponding cell of the in focus image, so as to move data in each cell to more finely align that data with the data in corresponding cells of the in focus image.

35. The apparatus of claim 32 wherein the coarse alignment further includes cropping the images so as to provide an image consisting of the overlapping regions of the defocused and in focus images, and wherein the fine alignment step includes dividing the cropped defocused images into the plurality of cells.

36. The apparatus of claim 33 wherein the plurality

of cells of the defocused image are moved relative to the in focus cells in a raster pattern, and correlation values for each movement in the pattern are obtained to enable the best correlation, and therefore the location of the in focus cell relative to the defocused cell which provides the best correlation to be obtained.

37. The apparatus of claim 36 wherein the correlation value for each cell is averaged with neighbouring cells so as to produce an average correlation factor.

38. The apparatus of claim 37 wherein the cells are warped in accordance with the correlation factors in order to move information within the cells so as to provide fine correlation of that information with the corresponding information in the in focus image.

39. The apparatus of claim 38 wherein the distortion is removed by the processor by a transformation obtained by obtaining data relating to a known reference sample, producing a transformation required to convert the detected data relating to the known reference sample to conform with the known reference sample, applying that transformation to the data produced by the detector so as to convert the data to thereby compensate for aberrations introduced by the magnetic lens.

40. A method of producing a phase image of an object using in focus and defocused images produced by a beam of electrons which transmit through or are reflected from an object, and in which the beam of electron is controlled to produce in focus and defocused images of the object, said apparatus including the steps of:

(a) removing distortions from the defocused images introduced into the defocused images when the defocused images are obtained by the control of the magnetic lens; and

(b) processing the in focus and defocused images to produce a phase image of the object.

41. The method of claim 40 wherein the phase image is produced from the in focus image and two defocused images of the object.

42. The method according to claim 40 wherein the step of removing distortions comprises performing a transformation on the defocused images to transform the defocused images to adjust for distortions introduced by the magnetic lens.

43. The method according to claim 42 wherein the transformation is obtained by imaging a known reference sample, producing the transformation required to convert the image of the known reference sample to conform with the known configuration of the known reference sample.

44. The method of claim 43 wherein the transformation is obtained by comparing the defocused image with the in focus image, and the defocused image is transformed so as to align data in the defocused image with corresponding data in the in focus image.

45. The method of claim 44 wherein the transformation comprises a coarse transformation in which the defocused image and the in focus image are overlapped and one of the images is moved relative to the other image to align data in the images, and then a fine alignment step in which the defocused image is divided into cells and each cell is compared with a corresponding cell of the in focus image, so as to move data in each cell to more finely align that data with the data in corresponding cells of the in focus image.

46. The method of claim 45 wherein the coarse alignment step further includes cropping the images so as to provide an image consisting of the overlapping regions of the defocused and in focus images, and wherein the fine alignment step includes dividing the cropped defocused images into the plurality of cells.

47. The method of claim 46 wherein the plurality of cells of the defocused image are moved relative to the in focus cells in a raster pattern, and correlation values for each movement in the pattern are obtained to enable the best correlation, and therefore the location of the in focus cell relative to the defocused cell which provides the best correlation.

48. The method of claim 47 wherein the correlation value for each cell is averaged with neighbouring cells so as to produce an average correlation factor.

49. The method of claim 48 wherein the cells are warped in accordance with the correlation factors in order to move information within the cells so as to provide fine correlation of that information with the corresponding information in the in focus image.

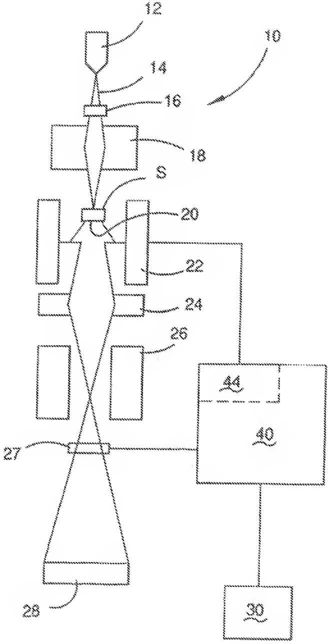

FIGURE 1

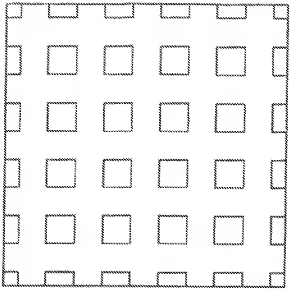

FIGURE 2

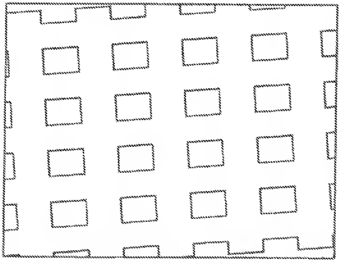

FIGURE 3

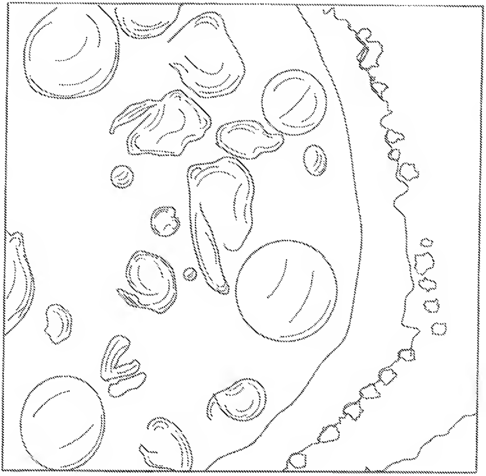

FIGURE 4

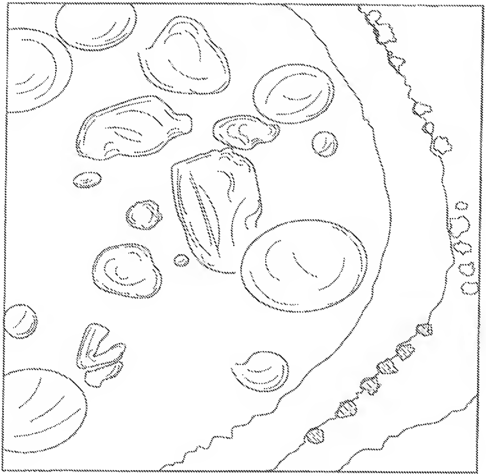

FIGURE 5

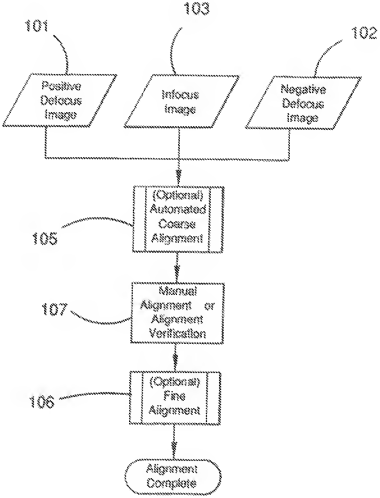
101
103
102
Positive Defocus Image
Infocus Image
Negative Defocus Image
(Optional) Automated Coarse Alignment
105
Manual Alignment or Alignment Verification
107
(Optional) Fine Alignment
106
Alignment Complete

FIGURE 6

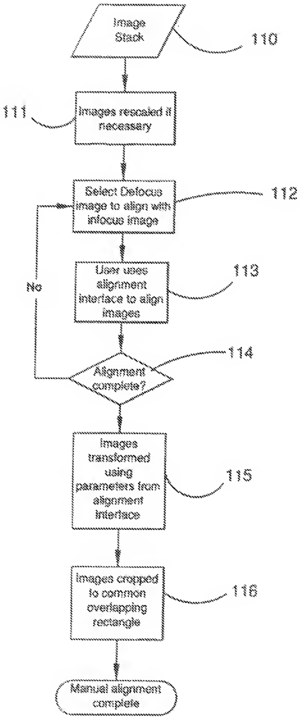
Image Stack
110
111
Images rescaled if necessary
Select Defocus image to align with infocus image
112
User uses alignment interface to align images
113
No
Alignment complete?
114
Images transformed using parameters from alignment interface
115
Images cropped to common overlapping rectangle
116
Manual alignment complete

FIGURE 7

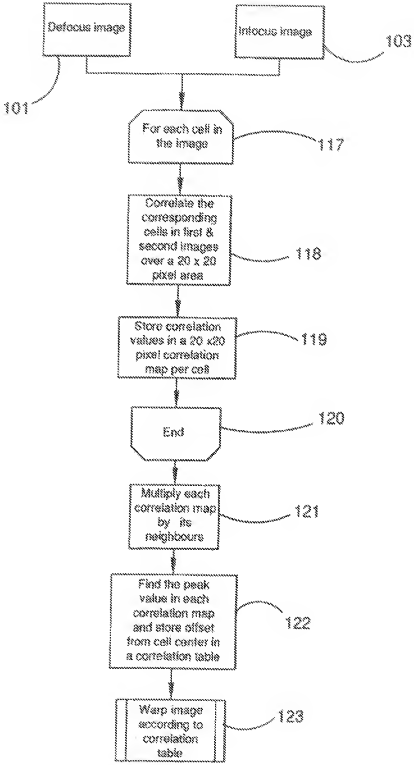
Defocus image
Infocus image
103
101
For each cell in the image
117
Correlate the corresponding cells in first & second images over a 20 x 20 pixel area
118
Store correlation values in a 20 x20 pixel correlation map per cell
119
End
120
Multiply each correlation map by its neighbours
121
Find the peak value in each correlation map and store offset from cell center in a correlation table
122
Warp image according to correlation table
123

FIGURE 8

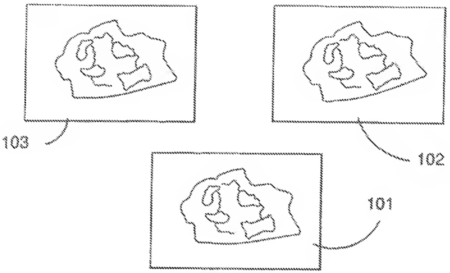

FIGURE 9

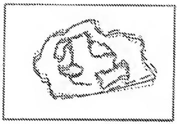

FIGURE 10

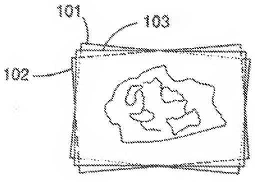

FIGURE 11

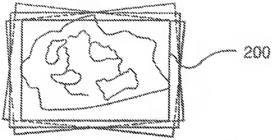

FIGURE 12

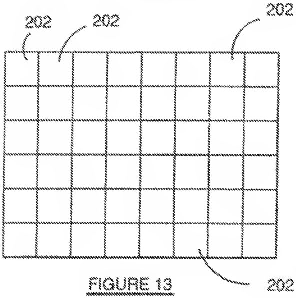

FIGURE 13

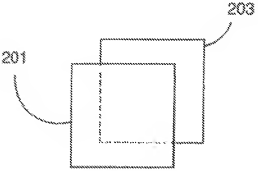

FIGURE 14

INTERNATIONAL SEARCH REPORT

International application No.
PCT/AU02/01019

A.	CLASSIFICATION OF SUBJECT MATTER
Int. Cl. [7]	H01J 37/22, 37/26

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
DWPI, JAPIO Keywords: electron microscop, electron beam; phase, wave front, wave field; defocus, out of focus, unfocus, nonfocus

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X Y	US 5134288 A (VAN DIJCK) 28 July 1992 Columns 1, 4-7, Figure	17-20, 23-25 21
Y	WO 00/26622 A (THE UNIVERSITY OF MELBOURNE) 11 May 2000 Pages 1, 26-27, 31, Figure 5, claim 1	21
A	Patent Abstracts of Japan, JP 05-217537 A (HONDA TOSHIKAZU AND TSUNO HISAYUKI) 27 August 1993 Abstract	1-49

☐ Further documents are listed in the continuation of Box C ☒ See patent family annex

* Special categories of cited documents:

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"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

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"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
12 September 2002	20 SEP 2002
Name and mailing address of the ISA/AU	Authorized officer
AUSTRALIAN PATENT OFFICE PO BOX 200, WODEN ACT 2606, AUSTRALIA E-mail address: pct@ipaustralia.gov.au Facsimile No. (02) 6285 3929	MICHAEL HALL Telephone No : (02) 6283 2474

International application No.

PCT/AU02/01019

This Annex lists the known "A" publication level patent family members relating to the patent documents cited in the above-mentioned international search report. The Australian Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent Document Cited in Search Report		Patent Family Member					
US	5134288	EP	472235	JP	4233150	NL	9001800
WO	200026622	AU	200015005	BR	9914976	EP	1127252
JP	5217537	NONE					

END OF ANNEX